INSULIN RELEASE FROM PIECES OF PANCREAS INCUBATED *IN VITRO* IN THE PRESENCE OF D-ERYTHROSE

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1. Introduction

A recent finding [1] that D-erythrose produces hyperglycemia in rats injected with the tetrose, led us to study the metabolism of this tetrose in the intact animal. Gluconeogenesis, glycogenolysis, increased glucagon secretion or activity, impaired insulin secretion or activity, other factors and combinations of those enumerated were considered as possible causes of the observed hyperglycemia. Preliminary experiments did not exclude the possibility that a low insulin level in the circulation may be the cause of the observed hyperglycemia [2].

It has been suggested that the "glucose-induced" insulin release is stimulated by glucose or a metabolite derived from glucose or other metabolizable sugars [3]. D-Mannoheptulose has been shown to cause a transient hyperglycemia in rats injected with the heptulose [4], to block the glucose-induced insulin release from pieces of pancreas [5, 6] and from isolated islets of Langerhans [7]. D-Mannoheptulose has also been shown to inhibit glucose phosphorylation and its inhibitory effect on insulin release has been attributed to its possible effect on glucose metabolism in the β cells [8]. On the other hand, it has been suggested that glucose *per se*, rather than a metabolite of glucose signals insulin release from β cells, and that mannoheptulose competes with glucose for "gluco-receptors" presumably located on the cell membrane [9]. It has also recently been found that D-erythrose inhibits growth of *Vibrio cholera* by inhibition of glucose transport across the cell membrane [10].

In this communication we report on the effect of D-erythrose on the *in vitro* insulin release from pieces of pancreas (cf. [6]).

2. Materials and methods

2.1. *Chemicals*

All chemicals were analytical or reagent grade. L(+)-lactic acid (Sigma Chemicals Co., or Analar, British Drug Houses), fumaric acid (99%, Matheson, Colleman and Bell), mono-sodium-L-glutamate (purum, Fluka), bovine albumin (powder, Fraction V from bovine albumin, Armour Pharmaceutical Co.) were purchased from the producers.

D-Erythrose was prepared from 2,4-*O*-ethylidene-D-erythrose [11] by hydrolysis with 0.1 N sulfuric acid, neutralization with barium carbonate, decoloration with carbon, and deionization with a mixed-bed ion-exchange resin. D-Erythrose was estimated by oxidation with periodate and measurement of the liberated formaldehyde [12].

D-Mannoheptulose was a gift of Professor E. Simon The Weizmann Institute of Science, Rehovot, Israel. The Insulin Immunoassay Kit was obtained from the Radiochemical Centre (Amersham, England). Crystalline beef zinc insulin was a gift from Eli Lilly and Company.

2.2. *Animals*

Pancreata were obtained from rabbits of both sexes, of unidentified ancestry. Rabbits (1.5–2.0 kg) were maintained on Amrod No. 935 Laboratory Animal Chow (from Amber Limited, Hadera, Israel) and had food and water *ad libitum*.

2.3. *Incubation media*

The basal medium used for the study of insulin release from pieces of pancreas incubated *in vitro* was the glucose-free, saline serum substitute, Medium I

Table 1
The effect of D-erythrose on insulin release from pieces of rabbit pancreas incubated *in vitro*.

μu insulin released/g/min		Sugars and other compounds tested in 2nd incubation
1st incubation	2nd incubation	
42 ± 12	58 ± 20	2 mM glucose (7)*
75 ± 10	1130 ± 149	15 mM glucose (33)
87 ± 21	285 ± 49	15 mM glucose + 30 mM D-mannoheptulose (18)
170 ± 57	1902 ± 373	15 mM glucose + 30 mM D-erythrose (12)
106 ± 26	1459 ± 277	15 mM glucose + 40 mM erythritol (11)
8 ± 4	903 ± 133	15 mM glucose + 30 mM D-erythronolactone (4)

* Figures in parentheses indicate number of tissue pieces studied.

(M I) of Krebs [13]. The medium also contained 1 mg/ml plasma albumin. Preliminary experiments showed that the omission of lactate from the medium had no effect on insulin release from pieces of pancreas incubated under the conditions used. The medium used in the subsequent incubations reported here was medium M I with no lactate (M Ia).

Sugars, polyols and other compounds tested for their effect on insulin release were dissolved in basal medium M Ia. The incubation media were prepared and gassed with O_2–CO_2 (95-5) before use. Insulin release from pieces of pancreas was followed under the same gas mixture.

2.4. *Procedure*

Rabbits, after an overnight fast were killed by a blow on the back of the head. The pancreas with the splenic horn was removed into 2 mM glucose, was freed of fat, mesentery and blood, and cut into 12–15 pieces 30–60 mg each. Each piece (no more than 10 pieces were incubated in every experiment) was transferred into a glass cylinder 35 X 60 mm o.d. containing 2 mM glucose (3 ml). The cylinder was fitted with a rubber stopper having an inlet and an outlet (3 mm o.d. stainless steel tubes, the inlet reaching about 0.5 cm above the buffer surface) for gassing. The cylinders were immersed in a thermostatic bath at 37° with shaking at 50–60 cyc/min, gassed (while being shaken), for 2 min and clamped. This preincubation lasted 1 hr. The incubation medium was removed with a Pasteur pipette and discarded. The pieces of tissue were then resuspended in 2 mM glucose (3 ml), the cylinders were stoppered, shaken and gassed for 10 min, at the end of which time the incubation media were removed and discarded.

The actual incubation started after this 10 min "wash". The pieces of pancreas were suspended in 2 mM glucose (3 ml), gassed while being shaken (2 min) and the incubation was continued for 30 min, after which the medium was collected and frozen.

The pieces of pancreas were then suspended in M Ia (3 ml) containing glucose and/or test compound, at concentrations indicated in table 1, gassed and shaken for 10 min and the medium discarded (second "wash"). The pieces of pancreas were then resuspended in the same medium in which they were washed (3 ml), shaken and gassed again (2 min). This second incubation also lasted 30 min, at the end of which the media were collected and frozen. The tissue was blotted with filter paper and weighed to the nearest 0.5 mg.

2.5. *Insulin analysis*

The amount of insulin released from the pieces of pancreas into the media was analyzed in duplicate by the double antibody method, with the Insulin Immunoassay Kit by reference to a standard beef insulin solution. Radioactivity of the precipitated complex was measured in a Packard-Tricarb liquid scintillation spectrometer. Insulin release was expressed as μu insulin released per gram pancreas per min ±S.E.M. The recovery of added insulin in this procedure varies from 60 to 96% [6].

3. Results

The results are summarized in table 1. As may be seen there was a marked individual variation in the

measured basal insulin release, from the pieces of pancreas of different animals suspended in 2 mM glucose (first incubation), but the response of the tissue to the sugars tested in the second incubation was unequivocal. The response of the tissue to 15 mM glucose in the presence and absence of D-mannoheptulose are as expected from earlier work [5, 6].

The results demonstrate that, under the conditions used, D-erythrose, as well as erytritol and D-erythrono-γ-lactone, have no inhibitory effect on glucose-induced insulin release from pieces of rabbit pancreas *in vitro*.

Incorporation of D-erythrose (30 mM) in the medium of the first as well as in the second incubation, thus extending the effect of the tetrose on the tissue for a longer time, did not alter the glucose induced insulin release [130.7 ± 28.5 versus 3111.9 ± 988.1 μu/g/min (n=14)].

4. Discussion

The D-erythrose-induced hyperglycemia was demonstrated in rats, whereas the effect of the tetrose on insulin release *in vitro* was studied with rabbit pancreas. It is assumed, however, that the mode of action of the tetrose is the same in both animals. Experiments with the intact rats as well as with rat liver preparations have shown that D-erythrose is reduced to erythritol and is also oxidized to erythronic acid [14] in the whole animal. For this reason the effect of these compounds on insulin release *in vitro* was included in our study.

The lack of response of pieces of rabbit pancreas to D-erythrose, erythitol and D-erythrono-γ-lactone, to the glucose-induced insulin release, and the hyperglycemia observed with intact rats injected D-erythrose, may be interpreted in several ways such as: (a) a metabolite of D-erythrose, other than erythritol or D-erythrono-γ-lactone, formed in the intact animal inhibits insulin release, (b) an inhibitor of insulin release, not derived from D-erythrose, is formed in the intact rat on administration of the tetrose, (c) in the intact animal D-erythrose, or its derivative, affects an organ other than the pancreas.

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